Effect of the magnetic field in HEK-293T line, with Gadolinium

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Introduction

There has now been an increase in the use of ions under the influence of electromagnetic fields in the health area. Paramagnetic ions, such as Gadolinium (Gd), move differently within a magnetic field. Consequently, Gd is useful as a contrast agent for radiology imaging [1]. For most materials, the magnetic field effect (B) produced by the orbital motion of the electrons is zero or very small. Paramagnetic particles have atoms with permanent magnetic moments. These moments interact with each other in a weak way and are oriented randomly in the absence of an external magnetic field. But, when placed within a magnetic field, the atomic moments are aligned with the B field [2].

Gadolinium-based contrast agents (GBCA) are manufactured by a chelating process. It is a procedure in which the large organic molecules form a stable complex around the Gd. The chelate reduces the possibilities of toxicity that could result from exposure to Gd by renal elimination [US Food and Drug Administration]. The GBCA's are approved by the Food and Drug Administration (FDA) for use with magnetic resonance as a contrast agent to provide improved imaging of organs and tissues.

In addition, Gd chelates can be used to identify macromolecules or cell populations of interest. Although Gd is not biocompatible, very little is known about its potential toxicity after cell deschelation [3]. Gd contrast agents are widely used because, they are considered safe and effective (>97.5 %). It has been used even in patients with impaired renal disease or chronic renal failure [4]. However, some patients treated with GBCA had a correlation with nephrogenic systemic fibrosis disease (NFS). This correlation was presented by the Danish Medicines Agency (DMA) and report by [5]. In addition, Gd has been found in the tissue of patients with NSF [6]. Apoptosis caused by radiocontrast may cause hyperosmolal cytotoxicity [7]. But, GBCAs are less cytotoxic than other agents, such as iodine [8]. The risks and concerns about Gd can be guided towards proliferation and cellular apoptosis, it is necessary to reveal how Gd affects the proliferation activity at the cellular level [9].

In this work, gadoterate meglumine was used under the influence of a magnetic field generated by a Rodin type coil. The protein profile, as well as the DNA integrity and the induction of apoptosis via Caspase 3 of the renal cells of the cell line HEK-293T exposed to magnetic field (B), meglumine gadoterate (Gd) were evaluated.

Materials and Methods

Human kidney embryonic cells, HEK-293T (ATCC® CRL-3216™) line, were grown in DMEM (GIBCO®) medium with 10% of SFB (GIBCO®) and 0.1% of solution Antibac-Antifun 100 x (Microlab, No. Cat. MC-130) using culture bottles of 25 cm^2 (CORNING). To obtain a monolayer with a confluence >80%, the cells were incubated as show in Table 1 at 37 degrees Celsius and a concentration of 5% CO_2.

Cellular monolayers were washed with 2 ml of NaCl (0.9 %) for 5 minutes with gentle agitation, supernatant was discarded. Afterwards, 1 ml of trypsin-EDTA 0.05% (GIBCO®) was added to cells and were incubated at 37 degrees Celsius for 5 minutes. Then 1 ml of complete DMEM medium was added to inactivate the trypsin. The cell suspension was removed to a 10 ml conical tube (CORNING) and bottles were washed using 1 ml of complete DMEM medium. Cells were harvested by centrifuged by 2 minutes at 1200 rpm at room temperature and the supernatant was discarded.

Cell pellet was resuspended with 8 ml of complete DMEM medium and was separated into volumes of approximately 1 ml in 8 microtubes (2 ml), resulting in 3 experimental groups and the control, each tube with its respective duplicate. Tubes were labeled as C1, C2, B1, B2, Gd1, Gd2, Gd+B1 and Gd+B2. Cell viability was measured before exposures using 10 µl of cell suspension, 10 µl of 0.4 % trypan blue (GIBCO®) with 80 µl of DMEM medium. Live cells were counted in hematocytometer.

To the Gd and Gd+B groups were added 15 mM Meglumine Gadoterate (30 µl of DOTAREM, Guerbet). Groups B and Gd+B were introduced inside a Rodin type coil for its exposure to a magnetic field. Exposure to magnetic field was carried out by 6 minutes intervals for 2 hours through an interface connected to a program generated in Simulink® of Matlab®, which made frequency changes (100, 800, 1500, 2450 y 2500 Hz, with a field strength between 4.13 y 1.13 mT). C and Gd groups were maintained without magnetic field at the same room and room temperature. After stimulation each pellet was resuspend-

ed and a sample of 10 µl was used to evaluate cell viability after exposition. The rest of the samples were centrifuged at 1200 rpm for 2 minutes, supernatants was discarded, and pellets were washed twice with 500 µl cold PBS. A set of the four experimental conditions (C1, B1, Gd1 and Gd+B1) was used for DNA extraction by standard methods. Another set (C2, B2, Gd2, and Gd+B2), was used for protein quantification and analysis by SDS-PAGE.

Table 1: Incubation time required according to cellular concentration

Inuculum conentration	Incubation time (hr)
250 x 10^5	24
200 x 10^5	48
100 x 10^5	72

To obtain a higher concentration of proteins in the samples; for each 100 µl of sample, 400 µl of cold acetone and 5 µl of protease inhibitor were added. The samples already mixed were placed on ice for 15 minutes. It was centrifuged at 14000 rpm at 4 ° C for 5 minutes. With the help of a micropipette, the supernatant was removed and only the tablet was retained. The tubes containing the samples were left open for 10 minutes for the evaporation of the acetone residues. To the tablets were added: 6 µl of 1 × PBS, 6 µl of loading buffer (added with bromophenol blue), 1 µl of protease inhibitor and 1 µl of β-mercaptoethanol. Once the gels were prepared and positioned, 25 µg of protein was loaded per lane. Electrophoresis was run at 70 V for one hour in a BIORAD vertical electrophoresis cell.

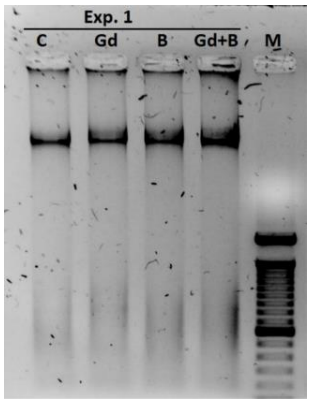

Figure 1: DNA extraction in 1% agarose gel. A representative gel was taken from expositions carried out, which was loaded with genomic DNA of control group and three experimental groups, and a 100 bp size molecular marker was used. There are no changes in DNA integrity.

The gels were stained with Coomassie blue, once stained, they were placed in solution to bleach with observations every 15 minutes. After 45 minutes, the image of the gels was taken with a ChemiDoc TM Touch photodocument, BIORAD Imaging System. The gels were placed for 30 more minutes in the solution to bleach, to take another image in the photodocument in a second time.

Results

To evaluate if experimental conditions induce any change in the DNA integrity of renal cells, a representative gel was done using each experimental condition. .Figure 1, shows no changes in integrity of DNA.

Polyacrylamide gels were loaded with 25 µg of protein extracted from control and experimental groups, and a molecular weight marker (M). No differences were observed in the protein profile of any experimental group compared to control group. The appearance or absence of a specific band was not detected in any of the experimental groups.

Based on the optical density obtained, concentration of active Caspase-3 was determined. The experimental groups (B, Gd and Gd + B) were taken as the dependent variables, and were compared with the control (C), with a $P < 0.05$, of which a significant difference was observed between the values presented in table 2, after performing a Fisher's test of the difference less significance. A significant increase of Caspase-3 was observed in groups treated with Gd and Gd+B, compared to control group. In addition, an increase of amount of Caspase-3 active was observed in Gd group and Gd+B, compared with group B. Also, a higher content of active Caspase-3 was observed in Gd+B group compared against Gd group.

Discussion

In our results, no increase or decrease in cell viability was found after having stimulated the HEK-293T renal cells, either with magnetic field (B), meglumine gadoterate (Gd) or a combination of both (Gd+B). With respect to DNA damage, it has been mentioned that damage is caused by exposing cells or organisms to fields that exceed safe limits, [10]. When adding iron ions or oxidative agents, DNA damage increases [11, 12].

However, in our work, degradation in DNA was not observed, but an increase in the induction of apoptosis was observed when exposing the renal cell cultures to Gd alone or together with field B [13, 14]. The field penetrates the nucleus without any attenuation, it reaches specific vulnerable sites that are sensitive to these fields and suffer damage, [15]. B fields can induce chromosomal abnormalities [16, 17], which could be increased by using a radiological contrast agent. Our results in the presence of B or Gd did not show changes in DNA integrity.

Table1: Fisher´s test of difference less significance, with a 5% significance for detection of active Caspase

Group	Significant D.	Critical V.	P
C,B	0.006	0.006	0.066
C,Gd	-0.013	0.006	0.001
C, Gd + B	-0.06	0.006	<0.0001
B, Gd	-0.019	0.005	<0.0001
B, Gd + B	-0.066	0.005	<0.0001
Gd, Gd + B	-0.047	0.005	<0.0001

The cytotoxicity of Gd can be one of the main sources that generate apoptosis in cell cultures. When Gd is combined with a B, it could behave like an ion and increase its toxic effect on cells [18, 19], since there are reports that mention that in the presence of only one field B, apoptosis is generated [20]. Ionic hyperosmolarity is also an important factor in the appearance of apoptosis. In conclusion after exposing renal cell cultures (HEK-293T) to a B with a frequency between 100 and 2500 Hz, with a field strength between 1.13 and 4.13 mT; to Gd at a concentration of 15mM; or to the combination of both (Gd+B), during a period of two hours, there was only an increase in the concentration of active Caspase-3 in both groups exposed to Gd and Gd+B, suggesting that this ferrofluid generates apoptosis in renal cells HEK-293T, either alone or in presence of a B.

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